Abstracts for Posters

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EFFECTS OF RATION LEVEL ON SELECTED PARAMETERS OF DISEASE RESISTANCE IN CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA)*

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Abstract

There is evidence that many of the specific and nonspecific host defenses of salmonids and other fishes are affected by specific dietary components. As part of a larger study funded by the Bonneville Power Administration to measure the effects of hatchery practices on physiological indices of captive salmonid broodstock, we developed a panel of hematological and immunological assays to monitor various indicators of the health of a fish's immune system. Analyses included measurements of hematocrit, leukocrit, differential leukocyte counts, plasma protein concentration, complement activity, and lysozyme activity. To monitor the non-specific cellular immune functions, leukocytes from the anterior kidney were analyzed to quantify the percentage of macrophages that would actively phagocytize bacteria, and the relative production of two bactericidal components by those cells, myeloperoxidase and the superoxide anion. We measured the effects of ration level (and consequently growth rate) on those indicators in chinook salmon. Groups of chinook salmon fingerlings were reared at 8 ℃-12 ℃ and fed a diet containing 54% protein and 7.4% lipid. Ration-level groups were 100% (fed to satiation), and 64% and 40% of the satiation quantity. Fish from each ration-level group were sampled for testing 27, 37, and 51 weeks after initiation of feeding with the experimental diets. As expected, during each sampling period fish in the 40% and 64% ration-level groups were smaller with regard to length and weight compared to fish in the 100% ration-level group ($p \leq 0.05$). Overall, there were no differences among the ration-level groups for the hematological analyses, except that leukocrit and plasma protein concentrations were higher in fish from the 100% ration-level group compared to levels in fish from the two other ration-level groups. The most interesting differences, however, were detected in the activity of the macrophages. The proportion of macrophages present in the kidney that would phagocytize bacteria was greater among fish from the group fed the 40% ration compared with fish from the group fed the 100% ration ($p \leq 0.05$). An effect of different rations on the production of myeloperoxidase by the phagocytic cells is also suggested by a trend toward more activity in phagocytic cells from fish fed the lowest ration. The increase in the percentage of phagocytic macrophages may indicate a state of greater activation in macrophages from fish reared at a non-satiating diet level. Conversely, the greater percentage of phagocytic cells may reflect the inability of the mature macrophages to emigrate from the hematopoietic tissues of the anterior kidney. This second explanation may be supported by the increase in circulating leukocytes in the fish reared at the higher ration levels. These results may be important to the fish culturist because macrophages are part of a salmonid's first lines of defense against infection by pathogenic microorganisms.

COLUMBIA RIVER FISH MARKING PROGRAM

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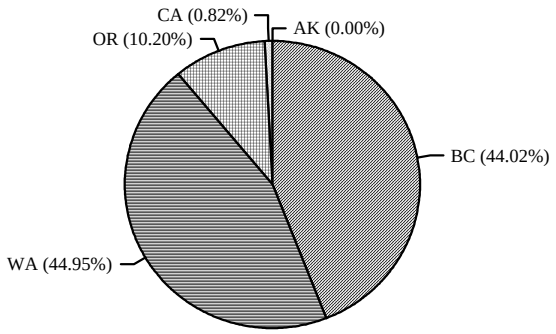
Abstract

Staff from the Columbia River Fisheries Program Office and contracted marking crews mark between six and ten million fish each year in the Columbia River basin. The types of marks used include fin clips, coded-wire tags, and PIT tags. The number of fish tagged at each hatchery varies with the objectives of the tagging study.

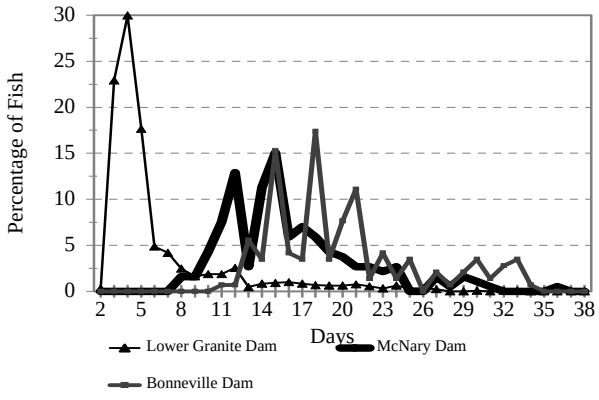
Coded-wire tags make it possible to recover information about where and when a group of fish are caught or return to the hatchery. Microscopic codes are etched into the wire when it is manufactured. The coded-wire is cut into one mm lengths and injected into the snouts of the fingerlings. Recovered tags can be decoded by technicians to provide information on the origin and age of the fish. The data can be used to estimate a number of statistics important to fishery managers, including overall survival, fishery harvest rates, and ocean distribution of the stock.

PIT tags are used to monitor the movement, passage and survival of migrating smolts and adult salmon and steelhead. The advantage of PIT tags is that they can be read or decoded without killing the fish. Information from PIT tags is used to manage flow and spill at dams; monitor hatchery and natural production programs; and evaluate predation, transportation, bypass, and survival.

Total Ocean Recoveries
Spring Creek NFH
Fall Chinook Salmon



Dworshak NFH Summer Steelhead - 1998
Outmigrant Travel Time



EVALUATION OF AMERICAN WHITE PELICANS AND BALD EAGLES AS VECTORS OF *MYXOBOLUS CEREBRALIS*

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Introduction

Whirling disease is caused by a parasite (*Myxobolus cerebralis*) that invades cartilage of young developing fish, encysting and becoming encased in bone as the fish develops. The parasite can move down stream during the free-swimming, triactinomyxon stage, or up-stream when an infected fish swims up-stream and dies. Transmission between watersheds can occur when the parasite is moved by man or animal, providing both the salmonid and oligochaete hosts are present in the receiving water. Determining the methods of transmission of the parasite, both within states and between states, is important to understanding distribution of the parasite.

Taylor and Lott (1978) demonstrated the possibility of bird transmission of *M. cerebralis* to previously uncontaminated waters. Black-crowned night herons (*Nycticorax nycticorax*) and mallard ducks (*Anas platyrhynchos*) were fed trout infected with *M. cerebralis*. The feces of the birds were placed in mud bottom troughs along with uninfected trout. The young trout eventually became infected indicating that spores survived the digestive tract of the two species of birds. The possibility of birds transmitting the spores around as they travel was demonstrated by Taylor and Lott (1978), but the overall importance of this mode of transmission is unknown. The length of time required by the bird to excrete all the spores might reflect the importance of this mode of transmission in the overall spread of the parasite. For example, if spores have all

passed through the birds in one day, the spread due to one meal may not be that significant. If the spores are voided by the birds over a longer period, transmission of the parasite could take place over much greater distances.

Range of American white pelicans is restricted to North America and nesting occurs primarily in the northern Great Plains. Pelicans also nest in smaller numbers in large lakes in western Canadian forests and marshes in the inter-mountain region of the U.S. Breeding habitat is associated with both lentic and lotic systems with shallow waters and high fish populations. American white pelicans nest colonially, mostly on islands that are inaccessible to terrestrial predators. American white pelicans are highly migratory, wintering in southern California, coastal mainland Mexico, the Gulf Coast of the U.S. and Mexico, and Florida. American white pelicans inhabit virtually every state west of the 100th meridian at some time during their annual cycle (Evans and Knopf 1993).

American white pelicans possess long pointed wings which are characteristic of highly migratory species (Rüppel 1977). In fact, migration routes and recoveries of American white pelicans banded in Yellowstone National Park cross virtually every major watershed in the western U.S. (Diem and Condon 1967). However, daily mechanics and rate of movement have not been recorded. If daily ranging behavior in summer ($\geq$105 km in one day) is indicative of capability during migration, American white pelicans may move in excess of 200 km per day during migration, potentially transporting agents of disease over large distances in one seasonal migration.

American white pelicans are opportunistic feeders, quickly cuing on temporal availability of food (Knopf and Kennedy 1980). Strictly piscivorous (Brouwer et al. 1994), American white pelicans feed on fishes usually 10 to 11 cm long, but will take individual fish up to 68 cm in length (Evans and Knopf 1993). Species commonly taken are carp (*Cyprinus carpio*) and creek chubs *(Semotilus atromaculatus*)(Knopf and Kennedy 1981) within 1 m of the surface, but crappie (*Pomoxis* spp.), suckers (*Catastomus* spp.), yellow perch (*Perca flavescens*), bullhead and catfish (*Ictalarus* spp.) are also taken (Wander 1981). Feeding method involves scooping fish dip-net fashion in shallow water (<100 cm), often after driving or surrounding fish by cooperative herding or occasional by pirating other piscivorous birds (Anderson 1991). Feeding activity occurs most frequently at night (O'Malley and Evans 1984, McMahon and Evans 1992), usually between 0001 and 0300 hrs with daytime hours spent loafing (O'Malley and Evans 1984). Foraging flights may extend up to 52 km from breeding areas in one day (Trottier et. al. 1980) and pelicans were found up to 308 km from the nesting colony in North Dakota (Lingle and Sloan 1980).

By the mid 1970's American white pelicans were considered to be declining range wide due to insecticides, shooting, and disturbance at breeding colonies (Udvardy 1977). By 1979, the U.S. population had declined nearly 9% (Sloan 1982). Coincident declines were noted in Montana, with populations decreasing between 51% and 75% from 1964 to 1972, allegedly a result of drastic fluctuations in water level (Sloan 1982). However, more widespread declines were

attributed to the effects of organochlorine pesticides on egg mortality (Lies and Behle 1966, Vermeer 1970). Populations appeared to rebound in the early 1980's (Roney 1982), reaching stability in the mid 1980's with only local fluctuations (Roney 1984). Population estimate in 1985 was 109,110 breeding birds, 59% in Canada and 41% in the U.S. (Siddle et al. 1985). In the 1990's, although no census data exist, populations appear to have increased dramatically. Where few American white pelicans were seen in the 1970's and 1980's in Montana (Missouri River and impoundments and other rivers in southwestern Montana), the species is now common and occasionally nuisance (e.g., Koonz 1981), especially as perceived by anglers (authors, pers. obs.).

Range of the bald eagle is restricted to North America. Historical distribution included every U.S. state and Canadian province plus portions of northern and eastern Mexico (Brown 1976). During the 1960's, when populations were depressed through the effects of the organochlorine pesticide DDT and its metabolites, breeding range shrank to include only remote, forested portions of the continent, mostly in Canada (Stalmaster 1987). Subsequent to the ban on use of DDT in the U.S. in 1973, bald eagle populations rebounded throughout their range (Grier 1982).

Most bald eagle nest sites are associated with large rivers, lakes, impoundments and coniferous and deciduous forests throughout the U.S. and Canada. Pairs nesting north of the Canadian border generally migrate to wintering areas in the Continental U.S. and Mexico. Bald eagles from Alaska generally move toward the coast of the state or at most to the northern coast of British Columbia for winter (Stalmaster 1987).

Creatures of aquatic environments, bald eagles occupy riparian or lacustrine habitat almost exclusively during the breeding season, but occasionally exploit upland areas for food and roost sites, especially during winter. Nest sites are most commonly distributed around the periphery of lakes and reservoirs $\geq$32.4 ha. in area and linearly along forested corridors of major rivers, usually within 1.6 km of shore (Wright and Escano 1986, Jensen 1988). Nest sites are usually as close to maximum foraging opportunities as possible (Harmata and Oakleaf 1992). Density of breeding pairs is highest along sinuous and braided sections of rivers and eutrophic lakes (Stalmaster 1987, Dzus and Gerrard 1993). Some pairs include both rivers and lakes in their home range. Bald eagles often forage year round near riffles, runs, and pools of rivers. On lakes and reservoirs, bald eagles, like American white pelicans, use shallow areas, associated wetlands, littoral zones with gently sloping shoreline and confluences of peripheral streams (Fielder and Starkey 1986, Caton et al. 1992). Estimates of home range size in the breeding season vary from 2 km^2 (Mattson 1974) to 234 km^2 (Yates 1989) but home ranges on rivers are more adequately represented linearly (length of river included) while minimum convex polygon (Mohr 1947) is more descriptive of home ranges on lakes. Accordingly, bald eagles incorporating only rivers in their range in Arizona and the Greater Yellowstone Ecosystem averaged 19 km and 7.6 km of river, respectively (Hunt et al. 1992, Harmata and Oakleaf 1992). Eagles incorporating only lakes in their home range had an average of 4 to 7 km^2 range in Saskatchewan (Gerrard et al. 1992) and 71.6 km^2 in Wyoming (Harmata and Oakleaf 1992).

Most young bald eagles leave natal breeding areas their first autumn, as do adults north of the 48th parallel. Bald eagles traveling to wintering areas scattered throughout the U.S. moved ≤50 km/day (Harmata et al. 1985, Hunt et al. 1992) but up 250 km/day in spring (Harmata 1984). Migrant bald eagles often follow spring spawning runs of local fishes, both altitudinally and latitudinally. During the remainder of summer, immature movements are often concentrated on larger lakes in their range. Bald eagle winter habitat is mostly associated with areas of ice-free water where fishes are available and/or waterfowl congregate (Snow 1973, Stalmaster 1987). Concentrations of vernal migrant eagles are small (<30) and dispersed and usually associated with waterfowl or small mammal concentrations because aquatic systems are usually frozen, limiting the availability of fishes. Food habits are eclectic, reflecting the opportunistic behavior of the species but fishes are clearly preferred (Snow 1973, Todd et al. 1982, Stalmaster 1987, Watson et al. 1991, Mersmann et al. 1992).

Other pathways of transmission may be involved in the spread of *M. cerebralis*, but only by evaluating each of these pathways can a comprehensive understanding of the of the spread of the parasite be developed. The rate at which birds pass excreta containing *M. cerebralis* spores may have implications on mode, distance, and distribution of the spread of whirling disease throught the western U.S. The objective of this study was to determine the passage time of a meal in the gastro-intestinal tract of piscivorous birds common to Montana waters. American white pelicans (*Pelecanus erythrorhynchos*) and bald eagles (*Haliaeetus leucocephalus*), two large (>4 kg) piscivorous birds frequently seen in western waterways, were chosen for study.

Methods

Two bald eagles and 4 white pelicans were evaluated. Bald eagles were captive birds used for educational and research purposes, were maintained at separate locations, and restrained with traditional falconry methods. Both were adult females. Pelicans were captured from a wild flock on Ennis lake, Madison County, Montana using a cannon net. Pelicans were restrained in individual cages at night. Cage dimensions were: floor, 122 x 122 cm; walls 122 x 117 cm; door- 107 x 122 cm. Floor was a 44 x 19 mm plastic coated, rigid steel mesh that allowed fecal material to drop through to a steel collection tray below. Walls and roof were a 5 cm diagonal, poly-netting. Because pelicans are gregarious and facilitate each other's behavior, cages were clustered to maintain close visual and auditory contact. During the day pelicans were at large for exercise in a barn with a fine beach sand floor. A variety of perches were available as were 3 large children's pools for swimming and hydration.

No *M. cerebralis* spores were used in this study due to containment concerns. Trace minerals were used as indicators of rate of passage, because concentrations can be easily and accurately measured. Two groups of captively raised rainbow trout (*Oncorhynchus mykiss*) were reared as food for the birds. The first group was fed a standard fish-meal based diet and were designated as "unmarked " fish. The other group was designated " marked" fish and received a diet containing krill meal which was high in strontium (Sr), Yttrium (Yt), and chromium (Cr) mineral

markers. Sr was selected as a marker because it deposits directly in bone of receipient fish. Thus, transit time of Sr through birds should be similar to *M. cerebralis* spores. Yt and Cr were chosen because they are commonly used for nutrition studies to determine nutrient digestibility or rate of passage. Yt and Cr were fed to marked trout in the oxide form and were indigestible, and thus remained in the intestinal tract of the trout. Sr, Yt, and Cr were analyzed in bald eagle fecal material but Sr and Yt were analyzed in pelican fecal material.

Pelicans were given a 30 day acclimation period to acclimate to the captive environment and facilitate normal metabolism prior to intiation of experimental feeding. Because eagle had been in captivity >3 years, no acclimation period was necessary. Unmarked fish were fed to eagles and pelicans for 3 days prior to feeding marked fish. Unmarked fish were then provided for 14 days. Eagles were fed to satiation once daily and excess food removed. Pelicans were fed daily in the evening after entering cages. Fecal material was collected from the substrate beneath perches (formica or plastic tarpaulin) bagged, dated, and identified by individual each morning, then substrate cleaned completely. Fecal material was analyzed for Cr, Yt and Sr using both Atomic Absorption Spectrophotometry (AAS) and Inductively-Coupled Plasma Spectrophotometry (ICP). Time from introduction of marked fish to maximum concentration of mineral markers in fecal material was determined as was the time from introduction of unmarked fish until minimum levels of mineral makers. These times were considered passage rates.

Results and Disscusion

Mean time to maximum level of mineral markers in bald eagle fecal material averaged 2.3 days after introduction of marked fish (Table 1). Mean time to reach minimum levels of mineral markers after introduction of unmarked fish was slightly longer (2.8 days). Times to maximum and minimum levels were not different among minerals and eagles ($F_s = 1.22$, $P = 0.36$). Time to maximum level of Sr in pelican fecal material after introduction of marked fish was not different between AAS and ICP analyses (Table 2). Mean time to maximum level of mineral markers in pelican fecal material averaged 2.3 days after introduction of marked fish. Unlike eagles, mean time to reach minimum levels of mineral markers after introduction of unmarked fish was slightly shorter (2.25 days). Times to maximum and minimum levels were not different among minerals and pelicans ($F_s = 1.00$, $P = 0.43$).

Results suggest that both species could excrete *M. cerebralis* spores from eating an infected fish over a 2-3 day period . Condition affects passage rate of food and birds not food stressed would tend to decrease transit time of digesta. Eagles used in this study were fed to excess each day and 3 of the 4 pelicans gained weight during the study, indicating subjects were in good condition. Thus estimates of period over which eagles and pelicans may expel *M. cerebralis* spores are conservative, and could be longer in wild birds. However, if infected fish are eaten just prior to extensive ranging flights (i.e., migration) coincident energetic stress could transport agents of whirling disease hundreds of miles from origin.

Because many factors contribute to incidence of whirling disease, (oligochaete hosts, exposure to triactinomyxons, and other unknowns), translocation of spores alone will not necessarily cause disease. Food habits of an avian species in a given watershed, food passage rate and migration strategies should be considered when evaluating the overall role of birds as vectors of fish diseases. Due to the number of different species of birds that may transport spores of *M. cerebralis* spores, migration distances and speeds of those birds, and rate of passage of digesta, attempts to control the spread of the parasite by controlling bird populations would be ill-advised, probably impossible, and most likely detrimental to the overall health of the ecosystem. These data indicate another natural mechanism for transmission of the parasite that should be considered when management plans are developed.

Table 1. Hours to maximum[1] and minimum[2] fecal marker level[3] of bald eagles fed marked and unmarked trout.

	Eagle 1		Eagle 2	
Mineral	Max.	Min.	Max.	Min
Strontium	48	72	48	72
Yttrium	48	72	72	48
Chromium	72	72	48	72
Mean	56	72	56	64
Mean by bird	64		60	
Eagle mean	62 hours			

[1] after consumption of marked fish for maximum fecal level of mineral
[2] after consumption of unmarked fish for minimum fecal level of mineral
[3] Inductively-Coupled Plasma Photometer, National Marine Fisheries Service, Seattle, WA.

Table 2. Hours to maximum[1] and minimum[2] fecal marker level of pelicans and fed marked and unmarked trout.

Mineral marker	Strontium				Yttrium	
Analysis Method	AA[3]		ICP[4]		ICP[4]	
Pelican	Max.[1]	Min.[2]	Max[1]	Min[2]	Max.[1]	Min.[2]
1	48	48	48	48	48	48
2	24	72	48	72	48	48
3	48	72	48	72	72	48
4	72	48	72	48	72	48
Mean	48	60	54	60	60	48
Method mean	54		57		54	
Mineral mean	55.5				54	
Pelican mean					54.7	

[1] hours after consumption of marked fish for maximum fecal level of mineral
[2] after consumption of unmarked fish for minimum fecal level of mineral
[3] Atomic Absorption Spectrometer, ACT Labs, Inc., Fort Collins, CO
[4] Inductively-Coupled Plasma Photometer, National Marine Fisheries Service, Seattle, WA.

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References

Anderson, J. G. T. 1991. Foraging behavior of the American white pelican (*Pelicanus erythrorhynchos*) in western Nevada. Colonial Waterbirds 14(2):166-172.

Brown, L. H. 1977. Eagles of the world. Universe Books, New York. 224 pp.

Brouwer, K., B. Hiddinga, and C. A. King. 1994. Management and breeding of pelicans, Pelecanus spp in captivity. International Zoo Yb. 33:24-39.

Caton, E., M. Donofrio and B.R. McClelland. 1989. Spring migration of a juvenile bald eagle from Glacier National Park: Habitat use and management recommendations for the Missouri River. U.S. Dept. Int., Nat. Park Serv., Res. Div. Glacier Nat. Park, W. Glacier, MT. 13 pp.

Diem, K. L. and D. D. Condon. 1967. Banding studies of water birds on the Molly Islands Yellowstone Lake, Wyoming. Yellowstone Library and Museum Association. Yellowstone National Park, Wyoming.

Dzus, E. H. and J. M. Gerrard. 1993. Factors influencing bald eagle densities in northcentral Saskatchewan. Journal of Wildlife Management. 57:771-778.

Evans, R. M., and F. L. Knopf. 1993. American white pelican (*Pelicanus erythrorhynchos*). *In:* The birds of North America. No. 57 (A. Poole and F. Gills, eds.). The Academy of Natural Sciences, Philadelphia, Pennsylvania USA and The American Ornithologists' Union, Washington, D.C. 24 pp.

Fielder, P. C. and R. G. Starkey. 1986. Bald eagle perch sites in eastern Washington. Northwest Science. 60:186-190.

Grier, J. W. 1982. Ban on DDT and subsequent recovery of reproduction in bald eagles. Science 218:1232-1235.

Harmata, A. R., and B. Oakleaf. 1992. Bald Eagles of the Greater Yellowstone Ecosystem: An ecological study with emphasis on the Snake River, Wyoming. Wyoming Game & Fish Dept., Lander. 368 pp.

Hunt, W. G., E. W. Bianchi, D. E. Driscoll and R. E. Jackman. 1992. Ecology of nesting bald eagles in Arizona. Report by BioSystems Analysis, Inc. to USDI, Bur. Reclamation, Lower Colorado Region, Boulder City, NV. Contract No. 6-CS-30-04470.

Jensen, K. C. 1988. Nest site selection by bald eagles in Montana. MS thesis. Montana St. Univ., Bozeman. 56 pp.

Koonz, W. H. 1981. White pelicans at nuisance grounds. Blue Jay 39(2):102-103.

Knopf, F. L., and J. L. Kennedy. 1981. Differential predation by 2 species of piscivorous birds. Wilson Bulletin 93:554-556.

Lies, M. F., and W. H. Behle. 1966. Status of the white pelican in the United States and Canada through 1964. Condor 68:279-292.

Lingle, G. R., and N. F. Sloan. 1980. Food habits of white pelicans during 1976 and 1977 at Chase Lake NWR, North Dakota. Wilson Bulletin 92:123-125.

Mattson, J. P. 1974. Interaction of a breeding pair of bald eagles with subadults at Sucker Lake, Michigan. MA Thesis, St. Cloud State University, St. Cloud, Minnesota. 51 pp.

McMahon, B. F., and R. M. Evans. 1992. Nocturnal foraging behavior in the American white pelican. Condor 94:101-109.

Mersmann, T. J., A. Buehler, J. D. Fraser and J. K. D. Seegar. 1992. Assessing bias in studies of bald eagle food habits. Journal of Wildlife Management. 56:73-78.

Mohr, C.O. 1947. Table of equivalent populations of North American small mammals. Am. Midland Nat. 37:233-249.

O'Malley, J. B. E. and R. N. Evans. 1984. Activity of American white pelicans (*Pelicanus erythrorhynchos*) at a traditional foraging area in Manitoba. Canadian Field-Naturalist 98:383-390.

Roney, K. 1982. 1980 census of Saskatchewan pelican and double-crested comorant colonies. Blue Jay 40:158-160.

Roney, K. 1984. 1982 census of Saskatchewan pelican and double-crested comorant colonies. Blue Jay 42:77-82.

Rüppell, G. 1977. Bird flight. Van Nostrand Reinhold Co., New York. 91 pp.

Sidle J. G., W. H. Koonz, and K. Roney. 1985. Status of the American white pelican: An update American Birds. 39:859-864.

Sloan, N. F. 1982. Status of breeding colonies of white pelicans in the U.S. through 1979 American Birds. 36:250-254.

Snow. C. 1973. Habitat management series for unique or endangered species. Bald eagle. U.S. Department of Interior, Bureau of Land Management. Report. No. 5, Denver Service Center, Federal. Center., Denver, Colorado. 58 pp.

Stalmaster, M.V. 1987. The bald eagle. Universe Books, New York. 227 pp.

Taylor, R.L. and M. Lott. 1978. Transmission of Salmonid Whirling Disease by Birds Infected with *Myxosoma cerebralis*. Journal of Protozoology. 25(1)105-106.

Todd, C.S., L.S. Young, R.B. Owen, Jr. and F.J. Gramlich. 1982. Food habits of bald eagles in Maine. Journal of Wildlife Management. 46(4):636-645.

Trottier G. C., R. J. Breneman and N. A. Young. 1980. Status and foraging distribution of white pelicans , Prince Albert National Park, Saskatchewan. Canadian Field-Naturalist 94:383-390.

Udvardy, M. D. F. 1977. The Audubon Society field guide to North American birds, western region. Alfred A. Knopf, Inc., New York.

Vermeer, K. 1970. Distribution and size of colonies of white pelicans, (*Pelicanus erythrorhynchos*), in Canada. Canadian Journal of Zoology 48(5):1029-1032.

Watson, J. W., M. G. Garrett, and R. G. Anthony. 1991. Foraging ecology of bald eagles in the Columbia River Estuary. Journal of Wildlife Management 55(3):429-499.

Wright, M. and R. E. F. Escano. 1986. Montana bald eagle nesting habitat macro-habitat description. U.S. Department of Agriculture, Forest Service., Missoula, Montana. 24 pp. plus appendices.

Yates, R. E. 1989. Bald eagle nesting ecology and habitat use: Lake McDonald, Glacier National Park, Montana. M.S. Thesis. University of Montana, Missoula. 102 pp.

CURRENT RESEARCH ON THE APPROVAL OF CHLORAMINE-T FOR USE IN PUBLIC AQUACULTURE

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Abstract

Chloramine-T is widely regarded as one of the most effective therapeutants used to control mortality in fish caused by bacteria associated with bacterial gill disease (BGD). However, the Food and Drug Administration (FDA) has not approved chloramine-T for use in any food animal, including fish. Therefore, before chloramine-T can be legally used in aquaculture without Investigational New Animal Drug (INAD) authorization, all technical sections necessary for approval of a New Animal Drug Application must be submitted and approved. Such technical sections include pivotal data on efficacy, target animal safety (TAS), human food safety, and environmental fate.

The USFWS Bozeman Fish Technology Center (BFTC) National INAD Office (NIO) is working cooperatively with FWS hatcheries, the USGS Upper Midwest Environmental Sciences Center, and Akzo-Nobel Chemicals, Inc. to generate the necessary data to gain FDA approval of chloramine-T for use in aquaculture. The BFTC has been responsible for conducting pivotal, near-pivotal, and ancillary chloramine-T field efficacy trials on salmonids, as well as pivotal TAS trials on various life stages of rainbow trout (Oncorhynchus mykiss). The efficacy of 12 - 20 mg/L chloramine-T has been tested on a variety of salmonids. The objective of each efficacy study has been to determine if chloramine-T treatments can control mortality in fish diagnosed with BGD. Results of efficacy studies have varied with respect to degree of "success", but nearly all treatments have been found to be efficacious. Results from TAS studies have also been promising. Regulated studies have been completed at two water temperatures for rainbow trout fry, fingerling and juvenile life stages. Groups of fish have been exposed to chloramine-T concentrations ranging from 0 to 100 mg/L for 3 hr in a standing bath on 3 alternate days at both 8 or 14^{o} C. Study results have shown that there is a positive correlation between toxicity of chloramine-T and both fish size and water temperature. Additional TAS studies are planned and this work should be completed before the end of FY2000. Final approval of chloramine-T will depend on a review and approval of the complete data package by FDA (which would include submissions for human food safety and environmental fate).

Background

Bacterial gill disease (BGD) is one of the most common diseases of hatchery reared salmonids (Bullock 1991) and is the cause of more fish losses than any other bacterial disease (Bills et al. 1988). In Ontario, Canada, BGD accounts for nearly 21% of all diagnostic submissions from fish

farms to the Fish Pathology Laboratory of the Ontario Veterinary College (Ferguson et al. 1991). Death is generally not a direct result of the infection, but is a consequence of the pathogen load. In the case of BGD, death is most likely the result of asphyxiation from lack of adequate oxygen exchange at severely congested gills. Stressors associated with intense fish culture may predispose fish to infection. Proliferation of gill epithelial tissue and subsequent loss of gill surface by clubbing and fusing of lamellae are often associated with BGD infections (Bullock 1990). The disease is characterized by an acute onset, flared opercula, increased branchial rate and decreased fright response. In addition, fish spaced equidistantly from each another, reject feed, and high mortality is observed (Lumsden et al. 1994; Lasee 1995). If BGD, which is horizontally transmitted, is not diagnosed and treated during the early stages of infection, thousands of fish may die within a 24-h period (Bullock et al. 1990).

Historically, a number of chemicals including benzalkonium chloride (available as Hyamine 1622 and 3500), diquat, and chloramine-T have been used to control mortality caused by BGD (Bullock et al. 1990). However, none of these available chemicals are approved by the U.S. Food and Drug Administration (FDA) to control mortality in freshwater fish caused by BGD or any other infectious pathogen. Because chloramine-T is generally regarded as the most effective therapeutant when salmonids have BGD (From 1980; Bullock et al. 1990), it has become the prime candidate for FDA approval as a bath treatment. Chloramine-T has been characterized as a non-selective sanitizing agent and has been shown to "clean up" gills infested with bacteria and coated with excess mucus. In order for a chemical such as chloramine-T to be approved for use in aquaculture, it must be proven to be efficacious in field trials, and pose no toxicological effects to exposed fish at the proposed therapeutic levels. Both efficacy and target animal safety studies, conducted to meet the FDA Center for Veterinary Medicine (CVM) minimum requirement for clinical and non-clinical field trials, are necessary components of a New Animal Drug Application submission. Efforts have been underway since 1997 to generate pivotal clinical field efficacy trials, and more recently, target animal safety (TAS) studies have been conducted to evaluate the toxicity of chloramine-T. Efficacy data generated to date, including data from both pivotal and non-pivotal trials, indicate chloramine-T treatment concentrations up to 20 mg/L to be efficacious while showing no indication of toxicological effects. Additionally, a series of target animal safety studies conducted at the NIO, which evaluated mortality among various life stages of rainbow trout exposed to $\leq$ 100 mg/L chloramine-T for 3 hours at 8 and 14^{o} C, also demonstrated no toxicity at 20 mg/L chloramine-T.

This presentation will summarize results of pivotal field efficacy and TAS studies conducted to date by the NIO and the U.S. Fish and Wildlife Service field stations. It will also describe other efficacy and TAS studies that still need to be completed, and an approximate time line anticipated for their completion and submission to CVM.

A total of 6 pivotal field efficacy trials have been completed. Species tested include Chum salmon (*Oncorhynchus keta*), rainbow trout (*O. mykiss*) and Apache trout *(O. apache*). Chloramine-T has been tested at concentrations ranging from 12 to 20 mg/L for 1 h using both flow through and standing bath treatment methods. Treatments were administered 3 times on

either alternate and consecutive days. Mortality was the primary response variable, and in nearly all cases mortality among treated groups was significantly less than among untreated groups. Table 1 summarizes efficacy data generated to date. Study reports for all studies have been completed. A report summarizing three of the reports was prepared by staff at the Upper Midwest Environmental Sciences Center and all four reports were packaged as a formal submission to CVM in August 1998 for review.

Table 1. Summary of pivotal studies conducted from 1997 through 1999. Table includes species test, treatment regime used, summary proportional mortality, whether differences were significant, how many of the quality criteria were met[1], and location of the study[2].

Fish Species	chl-T conc.	treatment frequenc	treatmen t	mortality T	U	Sign. diff.	Q. C.[1]	Study Location[2]
RBT	15	alternate	flow	56.8 %	65%	Yes	11 of 12	Neosho
CHS	12	alternate	standing	8.6%	97.6 %	Yes	12 of 12	Quilcene
APT	12	alternate	standing	30%	98.1 %	Yes	12 of 12	A-W C
RBT	13.2	alternate	standing	6%	25.6 %	Yes	11of 12	Hotchkis s
APT	20	alternate	standing	8.7%	98%	Yes	12 of 12	A-W C
RBT	20	consecuti ve	standing	5%	7.2%	No**	11of 12	Jones Hatchery

[1] Quality Criteria include the following: (1) disease identification; (2) data collected through the epizootic; (3) use of controls; (4) absence of known secondary disease agent(s); (5) quality control of data; (6) no concomitant therapy; (7) blinding techniques used; (8) randomization procedures used; (9) analytical confirmation of dosage; (10) replicates; (11) treatment consistent with proposed therapy; and (12) fish from a single test lot.

[2] Location of studies: Neosho National Fish Hatchery, Neosho, MO; Quilcene NFH, Quilcene, WA; Alchesay - Williams Creek NFH Complex, Whiteriver, AZ; Hotchkiss NFH, Hotchkiss, CO; Jones Hatchery, Hagerman, ID.

** There was no significant difference in mortality between treated and untreated groups. However, there was significant differences between the 2 groups of fish in the amount of bacteria present on gills.

A report will be prepared summarizing the remaining three study reports and will soon be submitted to CVM along with the following draft label claim:

Draft Label Instructions
For use on all salmonids susceptible to bacterial gill disease.

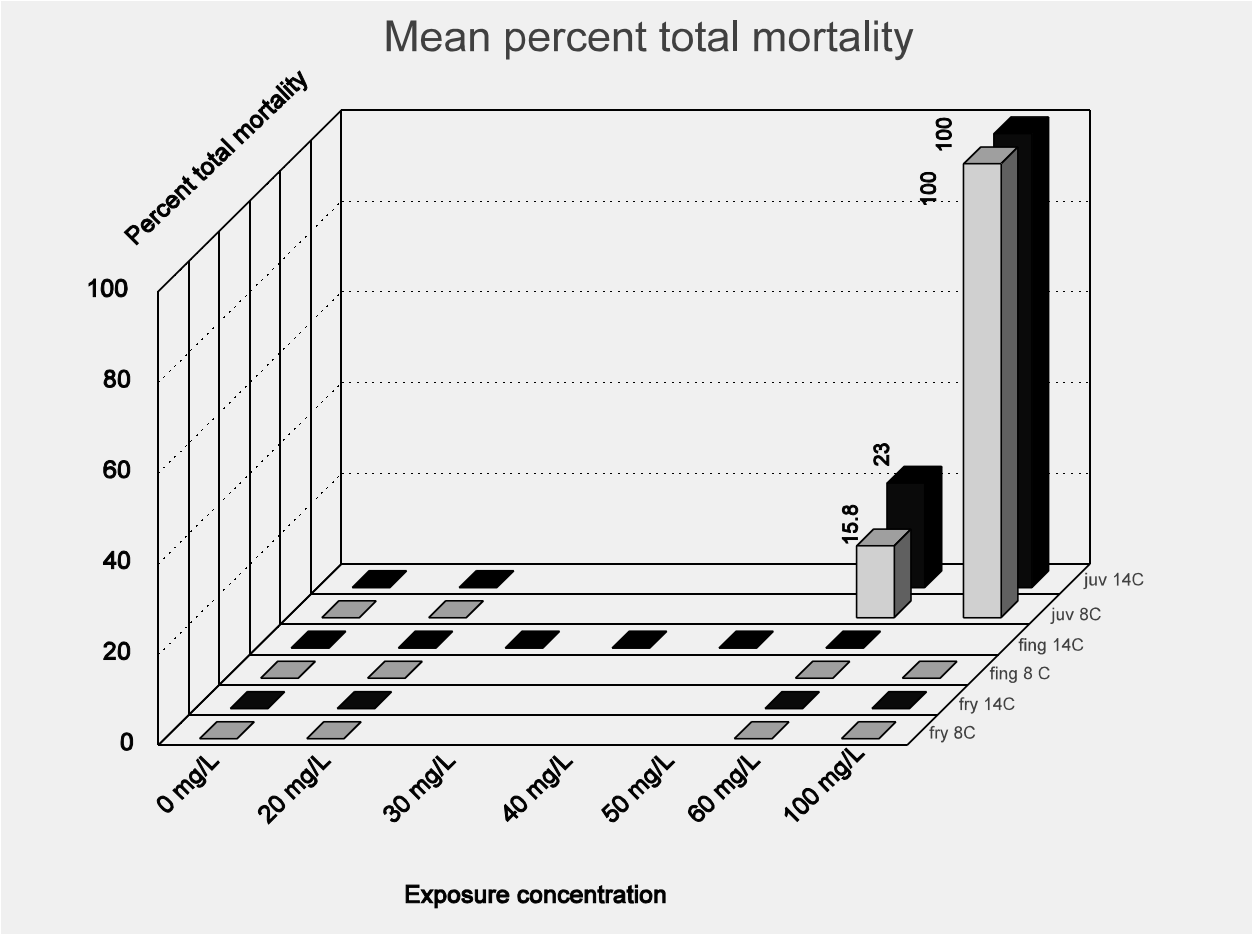
Indications: For the control of mortalities caused by bacterial gill disease.

Directions for Use:

> **Disease Control:** Treat fish at between 12 and 20 mg/L chloramine-T, in a 1 h standing bath or flow through immersion treatment, no more than once per day, on alternate days for a total of no more than three treatments per epizootic.

Current plans call for one additional pivotal efficacy study to be conducted at the Alchesay - Williams Creek NFH. The study will evaluate the efficacy of 10 mg/L chloramine-T administered for 1 h on three alternate days to control mortality in rainbow trout caused by BGD. If the study is successful, the directions of the proposed label will be modified to allow use of chloramine-T at concentrations between 10 and 20 mg/L.

The NIO has also initiated a series of TAS studies on various life stages of rainbow trout. Requirements for TAS studies are more stringent that for efficacy studies, as all TAS studies must be conducted according to Good Laboratory Practice standards. To date, studies have been conducted on fry, fingerling and juvenile rainbow trout at both 8 and 14^{o} C. Fish were exposed to chloramine-T concentrations ranging from 0 - 100 mg/L for 3 h in a standing bath on three alternate days. Results are summarized in Figure 1. In summary, there appears to be a positive correlation between chloramine-T toxicity and both age of fish and water temperature. Older fish were more sensitive to chloramine-T toxicity, and the chemical appears to be more toxic at higher water temperatures. A number of additional studies remain to be conducted. Included is a study to determine the toxic threshold of chloramine-T to fingerling and juvenile rainbow trout when tested at 14^{o} C. These life stages will also be used in a study to determine based on histological evaluation whether damage occurs to external surfaces (e.g., gills, skin, eyes) and/or internal tissue (kidney) when fish are exposed to sublethal concentrations of chloramine-T. If damage does occur to these tissues, then it will be important to determine whether the damage is reversible. Study plans also include the testing of adult rainbow trout at various concentrations of chloramine-T at two temperatures. It is anticipated that all above mentioned studies will be completed during the next 6 to 8 months. Analysis of data and evaluation of tissue by a certified histopathologist will likely require an additional 4 to 6 months. Reports will be prepared summarizing results from all studies, and a complete data package of this work will be submitted to CVM before the end of CY2000.


Mean percent total mortality
Percent total mortality
100
80
60
40
20
0
15.8
23
100
100
juv 14C
juv 8C
fing 14C
fing 8 C
fry 14C
fry 8C
0 mg/L
20 mg/L
30 mg/L
40 mg/L
50 mg/L
60 mg/L
100 mg/L
Exposure concentration

WASHINGTON STATE WARMWATER GAMEFISH ENHANCEMENT PROGRAM

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Abstract

The Washington Department of Fish and Wildlife's "warmwater" game fish enhancement program began in 1997 at the request of fishing organizations interested in bass, walleye, and other warmwater fish species that are found in Washington.

The enabling legislation authorized WDFW to fund the program through a $5 fishing license surcharge. Today the enhancement funds come from a share of the freshwater fishing license dollars. Prior to 1999, adult anglers paid $17 for a fishing license and an extra $5 to fish for bass, channel catfish, crappie, tiger musky, and walleye. Starting in 1999, adult anglers pay $20 for a license that covers all freshwater fishing.

Goals and Objectives of the Warmwater Enhancement Program

The Warmwater Gamefish Enhancement Program's goals is to increase opportunities to fish for and catch warmwater game fish. To do so, the program emphasizes field activities and the use of cooperative groups and volunteers whenever possible to minimize costs and gain the greatest return for the investment. The program also ensures no negative impacts to conservation of native anadromous species or other native species of fish and wildlife

Initial objectives and tasks included:

* Develop new lakes and ponds for warmwater fishing
* Provide more public access to existing warmwater fisheries
* Purchase and produce warmwater fish for stocking
* Develop and maintain an urban component to the program
* Evaluate new species and strains for introduction to state waters
* Use lake rehabilitation to improve warmwater fishing
* Use a variety of habitat-improvement projects to enhance warmwater fish populations
* Prevent the importation of deleterious exotic fish species

Accomplishments to date:

In the first two years of the Warmwater Gamefish Enhancement Program, WDFW has:

* Stocked more that two million bass, walleye, tiger musky, channel catfish, crappie and bluegill sunfish into more than four dozen Washington lakes, ponds and reservoirs.
* Conducted biological investigations on more than 40 Washington lakes to determine the best methods to enhance fishing in these waters.
* Rehabilitated three lakes to remove undesirable fish populations, and restocked these lakes with more desirable warmwater game fish species.
* Placed artificial habitat structure into five waters to enhance them for warmwater fish.
* Completed planning and improvements to provide more public access on over two dozen state waters, including fishing piers, a new public access on Sprague Lake, and other similar projects.
• Initiated research to determine how to enhance warmwater fishing without adversely impacting native fish and wildlife populations.
* Established two new juvenile fishing waters for warmwater game fish.
* Increased efforts to protect Washington's freshwater ecosystem from deleterious exotic species such as zebra mussel.
* Funded a major research effort to determine the impact of warmwater fish on various species of native fish and wildlife.
* Provided funding to complete construction of the new Rod Meseberg Warmwater Facility.

Future Program Development:

Expansion of the Rod Meseberg Warmwater Facility

Acquire and develop new nature rearing sites around the state for production.

Utilize existing local stocks for captive brood stock.

EVALUATION OF HATCHERY OUTPLANT RELEASE STRATEGIES FOR SPRING CHINOOK SMOLTS IN THE TUCANNON RIVER

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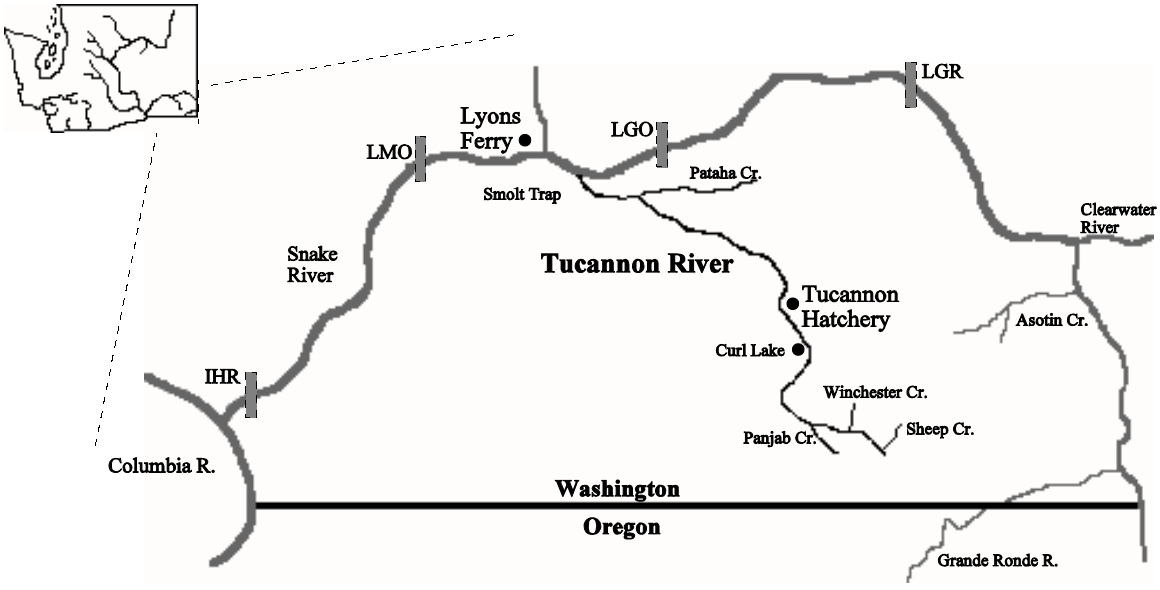
Background

With completion of the four Lower Snake River Dams, hatchery mitigation facilities (Lyons Ferry Hatchery-LFH, and Tucannon Hatchery-TFH) were constructed/modified to compensate for losses of returning adult salmon to the Snake River. One objective of these hatcheries was to compensate for the loss of 1,152 adult Tucannon River spring chinook salmon. In 1985, the WDFW initiated a hatchery supplementation program for spring chinook by trapping returning wild adults at TFH (Rk 58). Trapped fish are hauled to LFH, spawned, the progeny reared for 10 months, then transported back to TFH for acclimation and release (132,000 smolts). Also in 1985, a hatchery evaluation program was started to: 1) monitor the wild spring chinook production in the river, 2) determine the success of the hatchery program in meeting the mitigation goal, and 3) document effects the program may have on the natural population.

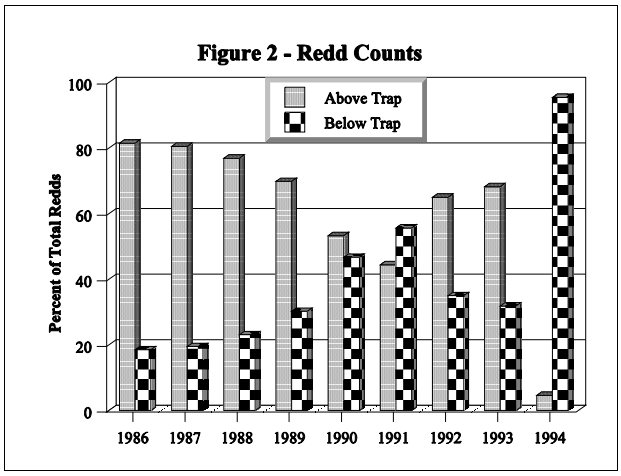
Figure 1 - Tucannon River Basin

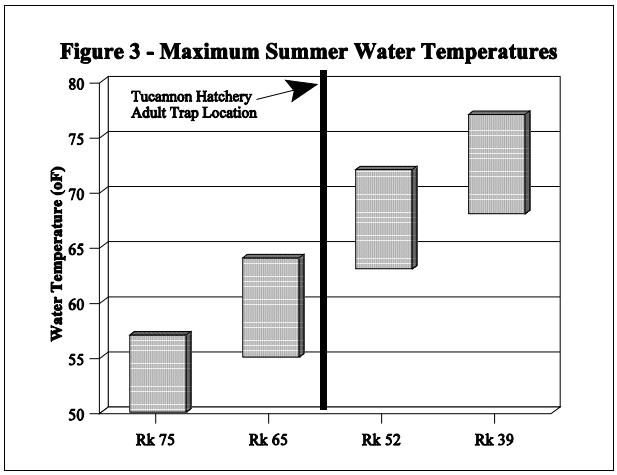


After nearly 15 years of monitoring, the evaluation program has determined that the wild population is below replacement, and the hatchery population returns at 2-3 times replacement. Therefore; the hatchery population is playing a critical role in maintaining the spring chinook population in the river, and presently is the only means of preserving the stock for the future.

Study Justification

After 10 years of monitoring redd numbers, a disturbing trend in the spawning distribution was observed (Figure 2). The number of redds above the TFH trap was declining, while redds below the trap increased. Summer rearing temperatures (Figure 3) and degraded habitat below TFH is not optimal for survival. Therefore; progeny survival from redds below the trap may be less. Temperature and habitat conditions above the hatchery are better. Reasons for the shift in spawning distribution were: 1) broodstock collections "mined" wild fish from the river which typically spawned 10-20 km above TFH, 2) juveniles were released at TFH, and returning hatchery adults appeared to be homing in on the hatchery, 3) high pre-spawning loss of hatchery origin fish passed above the TFH trap reduced the initial potential of more redds above the trap, and 4) a migration barrier (weir) to some fish caused by the TFH trap.





For those reasons, increasing redds below TFH and potentially reduced survival of juveniles forced the hatchery program to examine its release strategy. It was reasoned that releases of smolts upstream of TFH would have to occur to reverse the shift in spawning distribution. Unfortunately, no permanent facilities existed upstream of the hatchery. Direct stream releases or releases from small portable acclimation ponds were then proposed, with the intent that adults would return to their approximate point of release. However, both strategies were questioned by management. It was feared that transporting smolts just prior to release would lead to unacceptably high mortality. However, with no other choices except to continue current releases,

managers decided to try both proposed release strategies with some fish, while maintaining a large traditional release (90,000) from TFH. The evaluation program was given the task to monitor all aspects of releases, downstream migration, and adult returns.

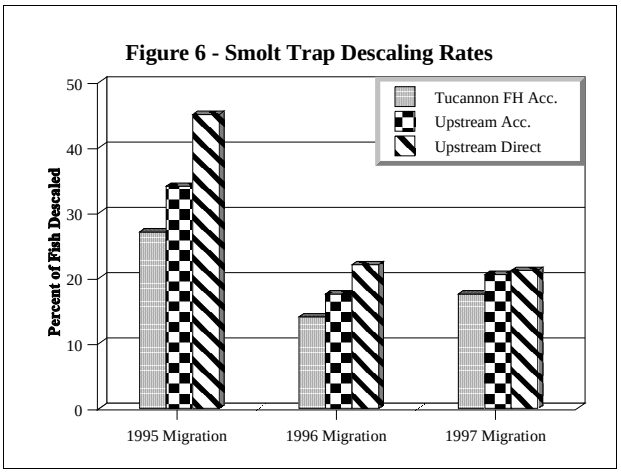
For the three study years presented (1995-1997 releases), there were three main release groups: 1) TFH - acclimated release , 2) Portable Ponds - acclimated release, and 3) direct stream release. All releases from portable ponds or directly to the river were released between Curl Lake (Rk 66) and Sheep Creek (Rk 84) (Figure 1). Fish released by the three methods were given unique Coded-Wire Tag codes for smolt-to-adult survival information.

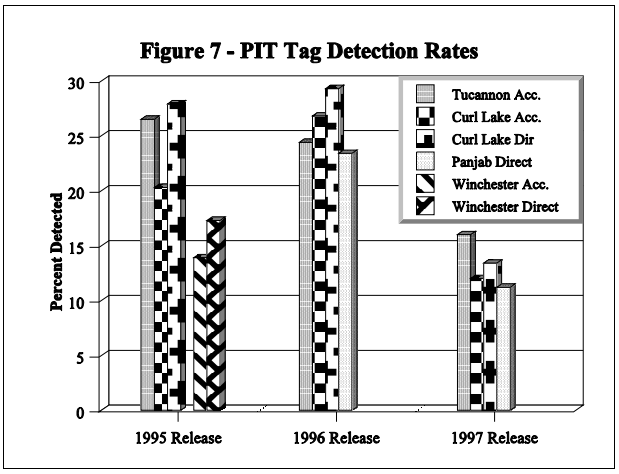
For planning the next years smolt release, evaluation methods were needed that could determine the success of each release strategy, without waiting for adult returns. Three techniques were used: 1) Blood Plasma Cortisol levels (stress indicator), 2) smolt trapping, and, 3) Passive Integrated Transponder (PIT) tagging. To determine origin of fish at the smolt trap, fish from the various release types were also tagged with a Visual Implant (VI) elastomer tag. Catches at the smolt trap could then be separated by release type.

Results: During all three release years, 20-60 fish per release group were sacrificed to determine stress levels based on blood plasma cortisol analysis. As expected, stress levels from fish transported and direct stream released were markedly higher (Figure 4) than the groups released from TFH or portable acclimation ponds. However, it was unknown wether this stress level would impact survival, or if the fish would recover and perform as well as the acclimated releases.

Following release, fish were captured in the smolt trap located near the Tucannon River mouth (Figure 1). Based on VI tags, survival and descaling rates were estimated for each release type (Figures 5 and 6). For two of the three years, direct stream released fish appeared to have higher survival rates than either acclimated group, though descaling rates were always higher. Descaling rates, like the cortisol results, were expected to be higher than the other groups as they were transported later, and were possibly more smolted. Only for the 1997 migration did estimated survival of the outplant groups appear to be less than the group released from TFH.

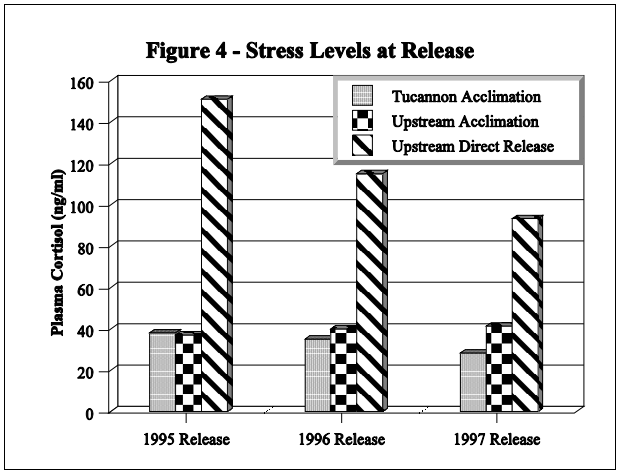
PIT tag detection percentages mimicked smolt trap survival estimates, and do not reflect the cortisol and descaling results. Direct stream released fish (released at Curl Lake, Rk 66) were detected at a higher rate than all acclimated group (TFH, Curl Lake, and Winchester) for 1995 and 1996 releases (Figure 7). However, not all direct stream released fish performed as well. Because PIT tagged fish could be isolated to release location and type (VI tags could not), we determined that releases in the upper most reaches of the river did not survive as well as groups released lower (1995 Winchester site release). Consequently, because of the PIT tag results, smolts releases in the upper reaches were discontinued after 1995.

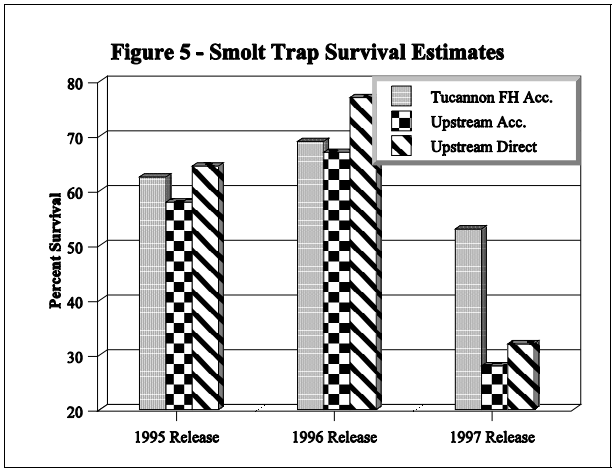




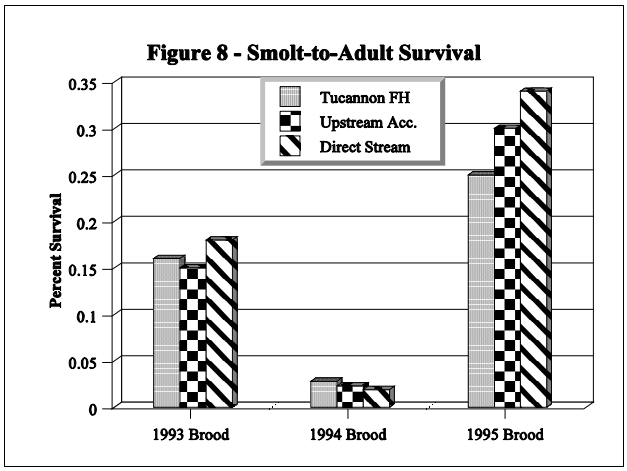
Discussion: Based the three years of data compiled, we were able to show overall that transporting smolts to small, portable acclimation ponds, or conducting direct stream releases was possible, and did not appear to result in a greater mortality than what was experienced from the standard release at TFH. Further, the evaluation on juveniles was valuable in quickly identifying optimal release points, allowing managers to make appropriate decisions for upcoming releases. What remained to be determined was wether adult survival would follow that which was documented for juveniles. Adult returns are nearly complete for the 1995-1997 release years (Figure 8). Based on the adult return data, no survival difference is apparent, and no one release particular strategy appears to be best.

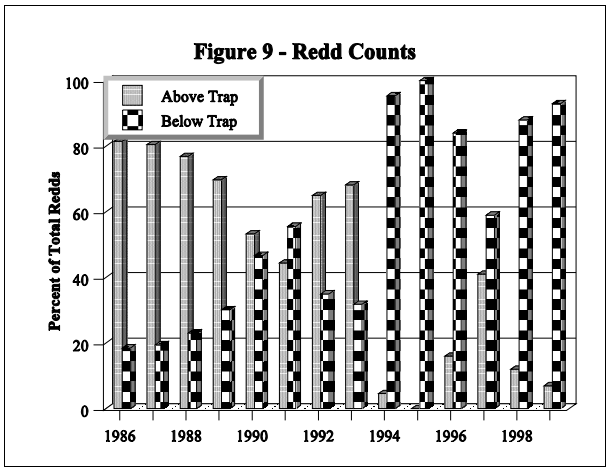
Based on initial study results, early adult returns, and other management changes within the basin, by the 1998 release managers had shifted to releasing 80% of the smolts upstream of the hatchery. By 1999, all releases occurred upstream of the hatchery (100% of the fish in Curl Lake





Acclimation Pond). Unfortunately, because adult returns have been low since 1994, nearly all returning fish (wild and hatchery) have been trapped at TFH for broodstock in an effort to preserve the stock, which has exacerbated the shift in spawning distribution (Figure 9). Moreover, since most of the fish have been collected, it has severely limited our ability to document the relative homing ability of fish released above the hatchery, to see if our efforts were worthwhile.





INTROGRESSIVE HYBRIDIZATION BETWEEN RAINBOW AND CUTTHROAT TROUT IN HENRY'S LAKE, IDAHO

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Abstract

For the past 30 years the IDFG has operated a spawning station on Henry's Lake to produce a trophy fishery of cutthroat Oncorhynchus clarki X rainbow Oncorhynchus mykiss hybrid trout. Eggs have also been collected for the production of pure Yellowstone cutthroat trout Oncorhynchus clarki bouvieri since the mid-1920's. Yellowstone cutthroat are produced to offset declines in natural production due to spawning habitat losses in Henry's Lake following construction of a dam at the outlet in 1924.

Each spring, spawning cutthroat return up Hatchery Creek to the spawning station on Henry's Lake. Returning cutthroat females are divided into two groups. Eggs from one group are fertilized with milt from selected returning cutthroat males. Eggs from the second group are fertilized with rainbow trout milt from Hayspur Hatchery, ID. The intentional hybridization program continues because of the large size and good condition the hybrids attain. The success of the Henry's Lake hybrid program as well as the future of the cutthroat fishery rely on the ability of managers to differentiate returning hybrids from returning cutthroat.

Male and female cutthroat trout and hybrids were sampled for two seasons as they returned to the hatchery. Sampled fish were identified as a cutthroat or a hybrid using several phenotypic characters such as pigmentation, size, and spotting pattern employed at the hatchery. Fish were subsequently examined using diagnostic allozyme loci, microsatellite fragment analysis, RFLPs of nuclear gene loci, and mitochondrial RFLPs. Additionally, cutthroat trout were collected and likewise examined from several tributaries of Henry's Lake to assess possible introgressive hybridization among those populations. Results indicate the phenotypic characters used to identify and select pure cutthroat females were not entirely accurate particularly during the later portions of the run.

THERMALLY INDUCED OTOLITH MARKING OF KOKANEE SALMON

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Abstract

The effect of thermal events on the otolith is nearly immediate, and a scheduled pattern of temperature changes will faithfully record a pattern on the otolith as a series of optically dense bands and intervening spaces (Volk, 1994). In order to evaluate the success of a kokanee (Oncorhynchus nerka) stocking program in Lake Pend Oreille, and otolith thermal mass-marking (T-marking) program was initiated at Cabinet Gorge Hatchery, Clark Fork, Idaho. Approximately 4.0 million kokanee were T-marked in 1996, 2.7 million kokanee T-marked in 1997, and 8.0 million T-marked in 1998. Trawl surveys on Lake Pend Oreille collected samples to be examined to determine the proportion of otolith marked hatchery fry versus non-marked wild fry.

NATIONAL INAD PROGRAM -- PARTICIPATION BY NON-USFWS ENTITIES ON USFWS INADS

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Bozeman Fish Technology Center, National INAD Office
Bozeman, MT

Abstract

In April 1998, the U.S. Fish and Wildlife Service (Service) took a major step in expanding both the scope and magnitude of its Investigational New Animal Drug (INAD) program by opening participation on Service INADs to state, tribal, university, and private aquaculture facilities. Although initial participation in the program was limited to folks in the States of Alaska, California, Idaho, Montana, Oregon, and Washington, in January 1999 the program was expanded to include participation by facilities from throughout the entire United States The new program has been termed the National INAD Program (NIP), although it is more commonly referred to as "Piggy-backing" on Service INADs.

The National INAD Program is an official program of the Service, and was established to facilitate consolidation of the INAD process, eliminate duplication of effort, reduce workloads and costs, ensure compliance with FDA guidelines, and ensure the submission of data that are necessary for future drug approval and drug labeling. The National INAD Program is a cost reimbursable program established under the direction and supervision of the Service's INAD Sponsor to ensure needed drugs, chemicals, and therapeutants are available to all aquaculture programs in the United States. The INAD Sponsor (through Cooperator funding) provides full administrative support for the office and staff of the National INAD Program.

To date, the NIP has been a resounding success. It has not only provided a mechanism for aquaculturists throughout the United States to continue to have access to needed drugs/therapeutants, but has also resulted in a the formation of a large, consolidated database of drug use efficacy data that can be used to support new animal drug approvals. Current participation includes 151 facilities located in 21 states scattered throughout the country. The NIP has experienced no regulatory or compliance problems associated with INAD drug use by NIP participants, and virtually all feed-back from participants has been positive. For additional information concerning the National INAD Program, please contact Dr. David Erdahl or Ms. Bonnie Elliott at (406) 587-9265 ext. 125 (Dave) or 136 (Bonnie).

REARING SALMON AND STEELHEAD IN HIGH-DENSITY MICHIGAN RACEWAYS: UMATILLA HATCHERY PROGRESS REPORT

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Abstract

Salmon and steelhead released in the Umatilla River have been reared in Michigan raceways at Umatilla Hatchery since 1992. These raceways produce more fish with less water by utilizing oxygen supplementation in a triple pass design. To test the effectiveness of these raceways, groups have been monitored to evaluate smolt quality, migration performance, and adult survival. When possible, these release groups were compared to groups reared in standard raceways. Subyearling fall chinook salmon have been reared in Michigan and standard raceways and our evaluation suggests the quality of fish produced in each system is similar. By rearing subyearlings in a Michigan series of raceways, we have produced approximately 950 fish/gpm compared to 320 fish/gpm in standard raceways. Recovery of marked juveniles at mainstem Columbia River dams suggests the migration travel time and survival of subyearlings reared in Michigan raceways and standard raceways was similar. Analysis of CWT data has been hampered by low recovery rates caused by a combination of poor migration survival out of the Umatilla River and poor ocean conditions. However, adult survival has been similar for both groups and suggests the MI raceways may be an effective fish culture technique for rearing subyearling chinook salmon. Because of a limited water supply, steelhead have been reared only in MI raceways at Umatilla Hatchery. However, production in a Michigan series of raceways has averaged 158 fish/gpm compared to an estimated production of 80 fish/gpm in standard raceways. Moreover, smolt-to adult survival of steelhead has been greater than 1% in some years and suggests these fish can be successfully produced in MI raceways. We are currently conducting studies to evaluate the maximum production of subyearling fall chinook salmon in MI raceways and plan to rear steelhead concurrently in Michigan and standard raceways.

A TRIAL OF THREE STARTER FEEDS ON STEELHEAD FRY AT DWORSHAK NATIONAL FISH HATCHERY

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Abstract

Steelhead at Dworshak National Fish Hatchery are reared from green eggs to smolts in a 12 month program which employs several reuse systems in order to rear the fish to a target release size of 180 to 220 mm in length. It is necessary to utilize reuse systems for the later takes of fish in order to heat the water so that these later fish will make size. The emphasis year round at Dworshak NFH is to maximize growth rates. Swim up steelhead fry at Dworshak National Fish Hatchery have traditionally been fed Bio Oregon's Bio diet starter feeds. Recently, Bio Oregon and Moore Clark feed companies have developed new starter diets. Bio Oregon has developed a floating flake starter feed and Moore Clarke has developed a crumble type starter feed. Both companies claim that these new feeds are an improvement over past feeds and will result in faster growth rates and improved feed conversions. A faster growth rate in the Dworshak nursery would result in steelhead fry being ponded outside at a larger size. This would shorten the time the fish would need to be on reuse during the winter months to reach release size. This would be advantageous since reuse is expensive to run and is also stressful on the fish. If the new feeds demonstrate improved conversion rates, it could translate to lower feed costs for the hatchery.

Twelve nursery tanks from BY99 spawning, take 7, were used. The tanks were split into three test groups of four tanks each. Bio Oregon starter diet was fed to the first group as the control. The other two groups were fed Bio Oregon flake starter diet and Moore Clark Nutra Starter. The tanks were stocked with 17,500 fish each. Beginning at swim up, a tub with a lid was placed next to each study tank. Each tank was fed from its corresponding tub. The fish were fed to satiation hourly, eight times a day, which is the standard feeding procedure for the Dworshak nursery fish. The feed was weighed into the tubs and remaining feed was back weighed at the end of the month to determine the total amount fed to each tank and calculate feed conversion. Approximately 25 fish were netted from each tank on three occasions and the length and weights were recorded. These data will be used to calculate mean length and weight for each tank, construct a length frequency distribution, calculate monthly growth rate as change in length (delta L), calculate feed conversion as pounds of feed fed per pounds of fish gained, and cost per pound of fish gained.

FISH COUNTING & BIO-MASS ASSESSMENT EQUIPMENT

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Abstract

The need for more accurate assessment of salmonid stocks in Northwest USA rivers is now heightened because of the Federal Endangered Species Act. National Marine Fisheries has developed guidelines for hatcheries releasing fish in rivers with ESA listed stocks. These guidelines now require hatchery managers to improve the accuracy of numbers and size of fish released from hatcheries in an attempt to lower stray rates and residualization (NMF section 10 permit, sub section 901, 902). Water Management Technologies, Inc., USA distributor of Vaki Fish Counting Technology offers fish counting and bio-mass assessment equipment to count adult Salmon migrations upstream through fish ladders, traps and cages as well as equipment to count volitional smolt migrations out of hatcheries, fish screens and dams. All products employ infra red technology enabling the equipment to operate day or night and in extremely turbid waters (2.3 inches on secchi disk).

The Vaki River Watcher provides accurate counting and assessment of salmon swimming through fish ladders, traps and cages. A record of the fishes' dimensions is taken for each fish. A silhouette image enables the viewer to determine hatchery versus wild fish by absence or presence of adipose fin. The River Watcher software distinguishes between fish passing upstream and downstream. The software also allows the user to program different size groups of fish and stores this information in the software's database for year to year comparison. Over 70 River Watcher's are installed and operating worldwide. The first unit was installed in 1993.

The Vaki Quattro Bioscanner is a high speed fish counter capable of counting 60,000 smolt per hour with +98 % accuracy. A Quattro is installed in a Washington State Fish Hatchery and will count smolt from a volitional release pond. All Vaki products are stress free counters that are fish friendly. Over 1100 Bioscanners have been sold since 1987.

"CAN-DO" HATCHERY OUTREACH PROJECTS

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Abstract

This poster presentation highlights a few innovative and cost-effective outreach projects that many hatchery stations could implement. These projects include: a high-tech touch screen information kiosk (Hagerman NFH); computer generated slide show for visitor orientation, student career fairs or any outreach event; a simple 2-sided newsletter produced in-house; "Fish Facts" Discovery Boxes and a "Wheel of Fish Fortune" activities for younger students. These ideas involve the help of outside partners, volunteers, students and creative hatchery staff. Supplies can be recycled materials, old or unused equipment; photos, surplus computer components, standard software programs, even natural items found around your station. Imagination and creativity are the only limits to your outreach programs, and of course the flexibility of hatchery managers is helpful. Each outreach project displayed has a cost breakdown, staff time involved, supplies and tools needed, anticipated audience, and learning goals/objectives.

OUTREACH LIFE CYCLE

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Abstract

As salmon and other fisheries concerns and issues become more prevalent and as our audiences grow in size and diversity, the need to provide hands-on learning experiences becomes critical. The only way to accomplish meaningful protection is to instill a sense of appreciation and understanding into every resident and visitor to our region. Innovative outreach, education, and interpretation is the key. Leavenworth National Fish Hatchery staff has developed creative year-round approaches to teach and share about Pacific Northwest fishery and watershed resources. Follow our "Outreach Life Cycle" and let us help you adapt some outreach tools and techniques to use at your site.

- *Kids in the Creek* — *-Create opportunities for high school students to jump into some waders and get wet! Join in a partnership with school district educators and teach watershed quality through a series of learning stations.*
- *Cascade Discovery Program* — *-Invite local school students to your site to explore your hatchery and the employees that work there. Incorporate mentorship programs into your daily routine.*
- *Wenatchee River Salmon Festival* — *--Share in the results of a successful curriculum that integrates quality natural and cultural resource education as it relates fisheries. Call us for a copy of this curriculum and adapt activities to your site.*
- *Hatchery Tours* — *--Share about hatchery operations and how they relate to the bigger picture of Pacific Northwest fishery resources by incorporating fun, hands-on environmental education activities.*
- *Open Houses* — *--Turn your **spawning days** into **learning days** for your community and local schools. Invite them to help out.*

- *National Fishing Week* — *--Using programs like "Pathways to Fishing," custom make a fishing event that teaches ethics in angling, fish biology, and habitat lessons.*
- *Cultural Exchange* — *--Get to know to your local tribes and help them share the significance of salmon in their cultures to your visitors.*

EPIDEMIOLOGICAL STUDIES ON IHN VIRUS IN SACRAMENTO R. CHINOOK

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Abstract

Infectious Hematopoietic Necrosis Virus (IHNV) infection has affected Coleman National Fish Hatchery (CNFH) juvenile chinook production since the 1940's. Annual fish losses in the tens of thousands is not unusual, however, overall production loss ranges from only 2 – 4 % due to the large size of the CNFH program (12 – 28 million chinook released). In spite of the epizootics at CNFH, the virulence of the Upper Sacramento R. strain of IHNV has been shown to be relatively low. Disease progression observed in the hatchery has not been replicated in experimental work. Risk assessment on the source(s) of infection and impact on natural fish from infected CNFH fish requires viral strain-specific data. These studies address the factors necessary for infection and progression to a disease state, incidence of infection in the natural population, distribution of infected hatchery fish following release, and the interactions of natural fish with released hatchery juveniles.

Sentinel fish studies in CNFH's water supplies have failed to detect IHNV in spite of concurrent epizootics within the production raceways. No infected natural chinook juveniles have been detected in 3 years of sampling in the Upper Sacramento River. Infected (but largely asymptomatic) CNFH out-migrants have been collected as far as 183 rm for several weeks post-release. Clinically sick fish rapidly shed up to 10^3 Plaque Forming Units (PFU) / mL into the water and mucus from these fish can contain $10^5 – 10^7$ PFU / mL. One minute water-borne challenges with as little as 100 PFU/mL results in infection; however, mortality is < 1%. Oral contact challenges also produce low rates of infection and little mortality. The incidence of infection (14%) and cumulative mortality (12.5%) from brief, low-level viral challenges was higher when fish were stressed post-exposure however, all groups remained asymptomatic throughout an 8 week study period.
The studies to date indicate that infection with Sacramento R. IHNV does not often lead to clinical disease in juvenile chinook and suggests that the ecological risk of infected hatchery fish may not be highly significant. Future study areas include the role of small birds in horizontal transmission within the hatchery, use of immunosuppressed fish for water supply sentinels, and a natural rearing experimental unit to examine sick hatchery fish interaction with natural chinook.Epidemiological Studies on IHN Virus in Sacramento R. Chinook.

EFFECT OF POVIDONE IODINE ON VIABILITY OF TYE TRIACTIHOMYXON STAGE OF *MYOXOBOLUS CEREBRALIS*

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Abstract

When fish eggs are collected from wild broodstocks and other sources outside the hatchery, the potential for bringing a pathogen into the hatchery is a serious concern to hatchery managers. As a precautionary matter, povidone iodine (1-ethyenyl-2-pyrrolidinone homopolymer and 1-vinyl-2-pyrrolidinone polymer iodine complex) is used routinely for disinfection of fish eggs in water that may potentially carry pathogens. Betadine® or PVP-Iodine is a commercial product that is 10% povidone-iodine (1% active iodine). The concentration of this stock solution typically used to disinfect salmonid eggs is 1% or 100 ppm of active iodine (Ross and Smith 1972; Amend 1974). While this concentration has been shown to be effective for controlling the majority of external bacteria and viruses in in-vitro tests (Amend and Pietsch 1972; Ross and Smith 1972; Chapman and Rogers 1992; Goldes and Mead 1995; Kumagai et al. 1998), the effect on Myxobolus cerebralis has not been tested. Vital staining has been useful for many applications, including the testing the viability of M. cerebralis (Markiw 1992).

A series of tests were conducted on triactinomyxons (TAMs), the infective stage of the salmonid parasite Myxobolus cerebralis, to determine the concentration of iodine needed to kill them. For each concentration, three to six replicate tests were conducted. For each test, 2 mL of the test iodine solution were mixed with 2 mL of TAM stock solution in a test tube and left at room temperature (15-20 C) for 10, 30 or 60 min. A minute or two before the time was up, the mixed solution was poured into a 10 um mesh filter to start filtering. At the allotted time, the filter retentate was rinsed with 20 mL of hatchery well water. This process took several minutes, after which 100 uL aliquots of retentate were transferred to 3-4 microscope slides. The slides were subsequently stained with 50-75 uL each of propidium iodide (52 mg/L) and fluorescein diacetate (100 uL of 5 mg/mL stock solution diluted with 8.3 mL hatchery well water). Control slides were made from the TAM stock solution and stained as noted above.

After incubation in a refrigerator for at least 45 min, the TAMs were observed by epifluoresence microscopy. The TAMs were classified as either red (dead), green (viable), or red and green (possibly viable).

The results of the iodine tests are presented in Table 1. Povidone-iodine concentrations of 50% or 5,000 ppm of active iodine for an hour were required to kill greater than 99% of the TAMs. This is 50 times the concentration typically recommended for treatment of fish eggs for bacterial and viral disinfection (McFadden 1969). The concentration of the stock solution was verified with a commercial colorimetric test. The same stock solution was used for all tests.

Clearly, higher concentrations of iodine are needed to adequately disinfect incoming water for M. cerebralis. The question becomes "How high can we safely go?" Amend (1974) tested the toxicity of Betadine and Wescodyne® (1.6% active iodine in the form of 9.1% polyethoxy polypropoxy polyethoxy ethanol-iodine complex, 8.74% nonylphenoxypolyethanoxyethanol iodine complex and 82% inert ingredients) to rainbow trout eggs. Amend (1974) found that toxicity was dependent upon pH and the stage of development of the eggs. At pH 6.9, the LC_{50} for active iodine was 1480 ppm in a 15 min treatment or 1050 ppm in a 60 min treatment of eyed eggs. If the solution was buffered, the LC_{50} of eyed eggs was increased to greater than 2000 ppm at either pH 7.0 or 8.0. When eggs were water hardened in iodine, 25 ppm of iodine was safe, but 100 ppm resulted in significant mortality. Leary and Pederson (1988) noted reduced survival in rainbow trout eggs water hardened in 1.24% Betadine buffered to pH 8.0. If eggs were allowed to water harden for 30 min, Amend (1974) reported no significant impacts on egg mortality, hatchability, or abnormalities at concentrations of 25, 100, or 200 ppm iodine. McFadden (1969) noted that up to 2.5% povidone iodine for 10 min was not toxic to eyed rainbow trout eggs, but concentrations of 3, 4, and 5% (300-500 ppm iodine) resulted in eggs surviving less than 24 h (no pH given). Alderman (1984) noted that concentrations of iodine from 75 to 200 ppm at pH values of 6.5, 6.75, or 7.5 for 10 min were safe for eyed Atlantic salmon Salmo salar eggs. For eyed rainbow trout eggs, Alderman (1984) tested concentrations of 50 to 4,000 ppm iodine for 10 min at pH levels from 3 to 8 and found that the LD_{25} was about 800 ppm at pH 6.0 and in excess of 3000 ppm at pH 7.0. For freshly fertilized rainbow trout eggs, Alderman (1984) found that mortality was also highly variable among females; 800 ppm iodine (either 10 min post-fertilization or after 30 min of water hardening) resulted in nearly complete mortality for eggs from some females and less than 10% for others.

Based upon the above research, achieving 5,000 ppm iodine without killing the eggs might be possible for eyed eggs at pH 8, but unrealistic for freshly fertilized eggs. Alternative chemicals for disinfection may be necessary. Glutaraldehyde was superior to iodine, chloramine-T, and sodium hypochlorite in treatment of plaice Pleuronectes platessa eggs (Salvesen and Vadstein 1995); concentrations of 400-800 mg/L for 5-10 min was recommended for Atlantic halibut Hippoglossus hippoglossus eggs whereas a shorter contact time (2.5 min) was recommended for Turbot Scophthalmus maximus (Salvesen et al. 1997). For treatment of largemouth bass Micropterus salmoides eggs, acriflavine (500-700 ppm for 15 min) was the disinfectant recommended by Wright and Snow (1975) over five other disinfectants.

Given the above data, exploration of alternative disinfectants may be necessary for prophylaxis against organisms such as M. cerebralis actinospores that may be carried with eggs into the hatchery. In the meantime, consideration should always be given to where water is dumped when bringing in eggs from the wild, taking care not to contaminate water supplies. When using iodophors, a fish culturist would also be wise to heed the recommendations of Chapman and Rogers (1992): use at least a 4:1 iodophor to egg volume ratio, make new solutions when treating multiple batches, and circulate the iodophor well during treatment.

Table 1. Mean percentage of non-viable, viable, and possibly viable triactinomyxons after exposure to various concentrations of povidone-iodine. Iodine concentrations are given as a percentage of the commercial povidone-iodine stock solution (10% povidone-iodine) and in active iodine concentration in ppm (given in parentheses). Control values were pooled.

Povidone-iodine concentration % (ppm active iodine)	Duration (min)	Dead (%)	Viable (%)	Possibly viable (%)	range of TAM numbers per replicate (n)
0.0 (0.0)		1.5	91.2	7.3	10-100 (24)
1.0 (100)	10	64.3	16.5	19.2	82-100 (3)
2.5 (250)	10	67.2	25.4	7.4	76-104 (4)
5.0 (500)	10	66.3	11.7	22.0	100-100 (3)
50.0 (5,000)	10	76.5	9.3	14.2	100-160 (3)
50.0 (5,000)	30	90.7	8.8	0.4	70-102 (3)
50.0 (5,000)	60	99.3	0.5	0.2	91-117 (6)

Literature Cited

Alderman, D.J. 1984. The toxicity of iodophors to salmonid eggs. Aquaculture 40:7-16.

Amend, D.F. 1974. Comparative toxicity of two iodophors to rainbow trout eggs. Transactions of the American Fisheries Society 103:73-78.

Amend, D.F., and J.P. Pietsch. 1972. Virucidal activity of two iodophors to salmonid viruses. Journal of the Fisheries Research Board of Canada 29:61-65.

Chapman, P.F., and R.W. Rogers. 1992. Decline in iodine concentration of iodophor during water hardening of salmonid eggs and methods to reduce this effect. Progressive Fish-Culturist 54:81-87.

Goldes, S.A., and S.L. Mead. 1995. Efficacy of iodophor disinfection against egg surface-associated infectious hematopoietic necrosis virus. Progressive Fish-Culturist 57:26-29.

Kumagai, A., K. Takahashi, S. Yamaoka, and H. Wakabayashi. 1998. Ineffectiveness of iodophor treatment in disinfecting salmonid eggs carrying *Cytophaga psychrophila*. Fish Pathology 33:123-128.

Leary, R.F., and J. Pederson. 1988. Effects of water-hardening eggs in a Betadyne or erythromycin solution on hatching success, development, and the genetic characteristics of rainbow trout. Population Genetics Laboratory Report 88/4, University of Montana, Missoula.

Markiw, M.E. 1992. Experimentally induced whirling disease II. Determination of longevity of the infective triactinomyxon stage of *Myxobolus cerebralis* by vital staining. Journal of Aquatic Animal Health 4:44-47.

McFadden, T.W. 1969. Effective disinfection of trout eggs to prevent egg transmission of *Aeromonas liquefaciens*. Journal of the Fisheries Research Board of Canada 26:2311-2318.

Ross, A.J., and C.A. Smith. 1972. Effect of two iodophores on bacterial and fungal fish pathogens. Journal of the Fisheries Research Board of Canada 29:1359-1361.

Salvesen, I., and O. Vadstein. 1995. Surface disinfection of eggs from marine fish: evaluation of four chemicals. Aquaculture International 3:155-171.

Salvesen, I., G. Øie, and O. Vadstein. 1997. Surface disinfection of Atlantic halibut and turbot eggs with glutaraldehyde: evaluation of concentrations and contact times. Aquaculture International 5:259-258.

Wright, L.D., and J.R. Snow. 1975. The effect of six chemicals for disinfection of largemouth bass eggs. Progressive Fish-Culturist 37:213-217.